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"Novel Vaccine Delivery Methods"

by

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2. Acknowledgments

The principal investigator of this project would like to acknowledge Marlene Snider, Catherine Ewen and Shawn Babiuk for their important contributions and dedicated work. The significant intellectual contributions, assistance and guidance of Dr. Marianna Foldvari from the College of Pharmacy and Nutrition are very much appreciated. In addition, I wish to thank the staff of the Animal Care Unit at VIDO for care and handling of the animals, Marilee Farrow, Michelle Balaski and Sherri Hueser for the preparation of grant proposals, and the entire VIDO Immunology group for their valuable discussions, suggestions and help. As a result of the generous support of the Saskatchewan Agricultural Development Fund we were able to obtain matching funds from the Alberta Agricultural Research Institute for this project.

3. Summary

Subunit vaccines are made of specific molecules from the disease causing organism. These vaccines are safe and cheap to produce. However, they elicit poor immune responses on their own and require the formulation with an adjuvant to be effective in protecting against disease. Most pathogens colonize or enter the host through the mucosal surfaces, therefore, the induction of a mucosal immune response is an important first line of defense in preventing enteric and respiratory diseases. Administration of vaccines through the mucosal routes (oral and intranasal) has been shown to provide specific protection from disease in numerous animal models including humans. New information and the availability of modern technology have facilitated the development of antigen delivery systems with the capacity to efficiently induce protective mucosal and systemic immunity against microbial pathogens.

4. Technical Report

4.1. Background

Current licensed vaccines cause excessive tissue damage resulting in economic losses due to the extra trimming of carcasses. In addition some of the vaccines now in use will no longer be allowed because of tissue residue problems. For example, a survey of injection site blemishes in beef cattle, conducted by the National Cattleman's Association (NCA), revealed that 15-20% of carcasses were affected. This high incidence of injection site blemishes in beef carcasses has been identified as a major concern for the meat packers, meat retailers, restaurateurs, and consumers. These blemishes included scars and fluid filled cysts that often required trimming of prime cuts from the rump region and adversely affected meat quality. It was estimated that the cost of this tissue damage was approximately $46 million US/year.

There is a clear need to develop alternative vaccine delivery systems that can maintain the health of livestock animals and minimize the use of antibiotics. It is essential that new vaccine strategies satisfy the livestock industry's mandate to produce a quality product while remaining competitive with other protein sources.

Most disease causing organisms enter the host through the mucosal surfaces (respiratory tract, alimentary tract and reproductive system) and therefore, the induction of a mucosal immune response is an important first line of defense in preventing diseases at these mucosal sites. The current vaccination strategies (intramuscular or subcutaneous routes) do not induce good immunity at the mucosal surfaces (lung and intestine).

Mucosal immunity is effective because it blocks the initial infection and dramatically reduces the development of disease. As a result of this research, there has been a great interest in developing vaccination strategies that preferentially induce mucosal immune responses for animal and human diseases.

We have shown that incorporation of vaccine antigens into liposomes have a significant impact on the enhancement of immune responses following systemic and

mucosal vaccination in mice. In order to apply this technology to large animals we need to develop methods to deliver intranasal vaccines.

In addition to intranasal vaccines, a novel approach to induce immunity has been described. This approach is based on the ability of vaccine antigens to induce immunity by the transdermal (through the skin) route. Dr. Marianna Foldvari at the College of Pharmacy and Nutrition at the U of S has been our collaborator in the area of liposome technology. She has developed expertise in the area of transdermal delivery for drugs and has patented this technology. She will be collaborating with us in developing formulations for transdermal delivery of protein and DNA vaccines.

Developing more effective vaccines that do not cause tissue damage will benefit the agriculture industry by reducing carcass trimming and reducing the use of antibiotics to treat disease. This will enhance the quality of beef produced and promote consumer confidence in the final product.

4.2. Objective

The objective of this project is to develop methods of vaccination that are not invasive and do not require a needle. Two methods of vaccine delivery are currently being assessed, intranasal and transdermal. We will design methods for delivering the vaccines as well as measuring their ability to confer protection against respiratory infections in various animal models.

4.3. Project Activities, Achievements and Publications

- Baca-Estrada, M. E., Foldvari, M., Ewen, C., Badea, I. and Babiuk, L. A. 1999. Effects of IL-12 on immune responses induced by transcutaneous immunization with antigens formulated in a novel lipid-based biphasic delivery system. Vaccine. In press.

- Maria E. Baca-Estrada, Marianna Foldvari, Catharine Ewen, Ildiko Badea and Lorne A. Babiuk. Induction of antigen-specific immune responses by transdermal delivery of antigens formulated in a novel lipid-based biphasic delivery system. Abstract presented at the Meeting of the American Association of Pharmaceutical Scientists. San Francisco CA. USA.

- Baca-Estrada, M.E., M. Foldvari, M. Snider and L. A. Babiuk. Effect of IL-4 and IL-12 liposomal formulations on the induction of mucosal and systemic immune responses to bovine herpesvirus type-1 glycoprotein D. 9th International Congress of Mucosal Immunology. January 1997, Sydney, Australia. Immunology and Cell Biology 75:A128, 1997.

- Baca-Estrada, M.E., Foldvari M., Snider M. 1999. Induction of mucosal immune responses by administration of liposomal-antigen formulations and IL-12. J. Interferon and Cytokine Res. 19:455-462.

- Papp, Zs., Middleton, D. M., Røntved, C.M., Foldvari, M., Gordon, J. R. and Baca-Estrada, M. E. Transtracheal administration of interleukin-12 induces intense neutrophil responses in the murine lung. J. Interferon Cytokine Res. In press.

4.4. Results

4.4.1. Mucosal immunization of cattle using a liposome based vaccine.

An experiment was designed to assess the ability of gD-liposome formulations to induce mucosal immunity in cattle. Groups of 10 animals were immunized intranasally with formulations containing placebo, gD alone (25 μg/animal) and gD in liposomes (25 μg/animal). The results of this experiment are shown in Fig. 1 and 2.

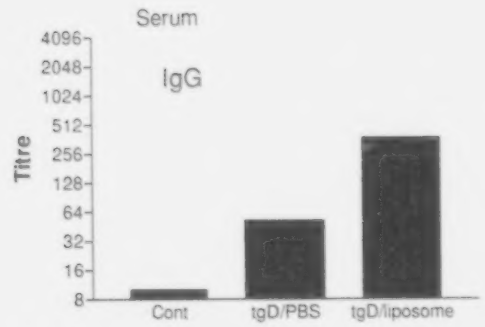

Fig. 1. Antibody response to gD in serum of immunized cattle. Animals were immunized intrnasally and boosted 21 days later. Serum samples were collected 10 days after the second immunization.

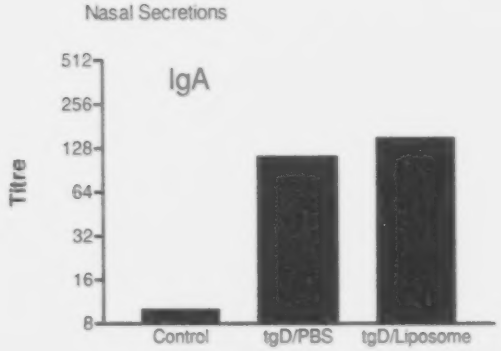

Fig. 2. Antibody responses in the mucosal secretions following intranasal immunization. Animals were immunized as in Fig.1. The gD-specific IgA titres were measured in nasal secretions.

Intranasal vaccination with liposome formulations induced significantly higher gD-specific IgG responses in serum. Interestingly, the gD-specific IgA responses in the mucosal secretions were similar between the groups immunized with gD or with gD in liposomes. These results indicate that induction of systemic (serum) responses was enhanced by liposome delivery, whereas induction of local antibody response was not. One explanation for these results is that the gD molecule is capable of binding to cells (it is a viral protein involved in cell adhesion) and therefore it is likely that gD could by itself bind to epithelial cells.

4.4.2. Intranasal Immunization of Cattle with Pasteurella haemolytica (Leukotoxin, Ltx) vaccine.

We demonstrated that intranasal administration of bovine herpes virus type-1 (BHV-1) vaccine (gD) formulated in liposomes was capable of inducing serum and mucosal specific-antibodies. These results led us to examine whether bacterial antigens such as the Ltx from *Pasteurella haemolytica* could induce similar immune responses.

Intranasal immunization of cattle with Ltx did not induce significant enhancement of specific antibodies when compared to the control group (fig 3). Interestingly, all animals including the control group, had high anti-Ltx antibody titres (8,000). These high background may have limited the ability of our vaccine protocol to enhance immune responses to Ltx.

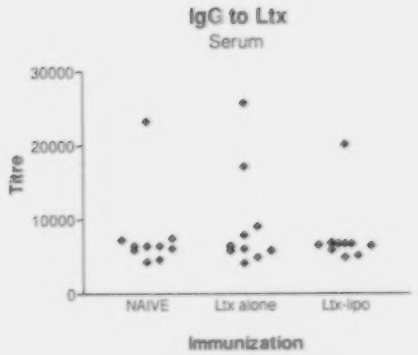

Fig. 3. Antibody responses in cattle following intranasal immunization with saline (naïve), Ltx alone and Ltx formulated in liposomes (Ltx-lipo). Each dot represents an individual animal. Note the high background level of anti-Ltx antibodies in the control group (mean of 7796).

4.4.3. *Intranasal Immunization of Sheep with Leukotoxin (Ltx) and Cholera toxin (CT) antigens.*

The following series of experiments were designed to evaluate the mucosal and systemic immune responses after intranasal immunization of sheep with two bacterial antigens, Ltx and CT. There are two important reasons to use sheep instead of cattle, one is the economical and the other is that both species have immune systems that are functionally and developmentally identical.

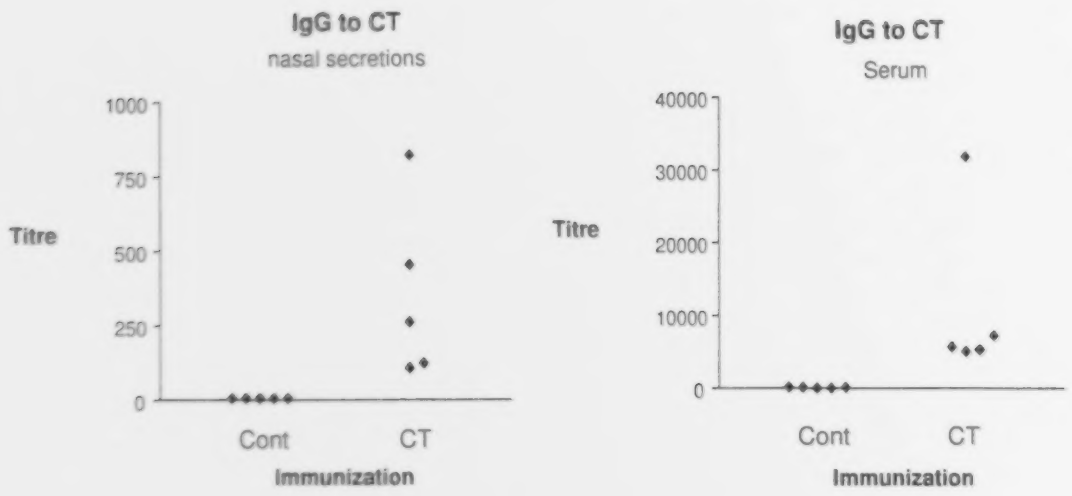

Fig. 4. Antibody responses to CT in the nasal secretions and in serum of sheep following intranasal immunization with CT formulated in liposomes. Each point represents individual animals.

Intranasal immunization with CT induced significant increase in mucosal (nasal secretions) and systemic (serum) specific IgG antibodies (fig.4). Due to technical problems we have been unable to measure CT-specific IgA. However, we have contacted researches in Australia and they have agreed to provide us with the reagents to measure sheep IgA. We expect that for the next report we will have this information.

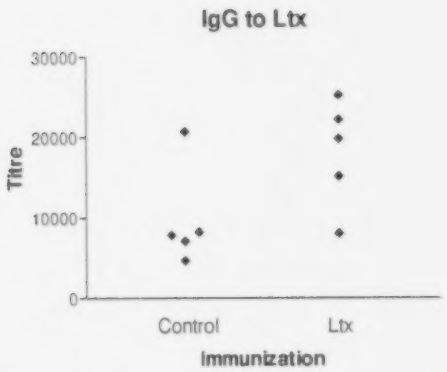

Fig. 5. Antibody responses in serum following intranasal immunization of sheep with Ltx. Immunized animals received 100 μg of Ltx formulated in liposomes. Each dot represents individual animals. High background levels of anti-Ltx are present in non-immunized control group.

Intranasal immunization of sheep with Ltx did not significantly enhanced the specific-antibody levels in serum (fig.5). Although the response was not statistically significant there was a trend that immunized animals had higher levels of Ltx-specific antibodies.

In conclusion, the results presented in this report illustrate that intranasal immunization of cattle and sheep can induce specific immune responses against the vaccine antigen. However, we also demonstrated that control non-immunized animals (cattle and sheep) contained high levels of anti-Ltx antibodies and that intranasal immunization with Ltx failed to enhance immune responses to this antigen.

4.4.4 Intranasal Immunization of Cattle with Liposome Formulations Containing gD and Quil-A as an Adjuvant.

We have previously shown that immunization of cattle with gD antigen formulated in liposomes induced mucosal immune responses against gD. As a second step in this immunization approach we designed an experiment to assess the effect of

Quil-A in enhancing the mucosal immune response using liposomes as delivery systems. The experiment was carried with calves from the Western Beef Development Center at the Termuende Farm.

The experimental design consisted of 5 groups of 6 animals each. The treatments consisted in: gD alone; gD in liposomes; gD + Quil-A and gD + Quil-A in liposomes. The negative control group received saline. All animals were immunized intranasally and 3 weeks later were boosted with the same formulation and by the same route. Ten days after the boost all animals were bled and the nasal secretions collected to measure the levels of gD-specific antibodies (IgA and IgG).

In this experiment the liposome-gD formulations did not induce a systemic or mucosal immune response. In addition, Quil-A had no effect in enhancing immune responses to gD. At the present time we do not have an explanation for the negative results of this experiments. We are currently assessing the feasibility of the intranasal route to induce immune responses in cattle. The methods to deliver the vaccine may have to be redesigned to allow better administration of the formulations.

4.4.5. Assessment of Transdermal Delivery of Antigens for Induction of Immune Responses in Mice.

One of the main objectives for this past reporting period was the evaluation of transdermal immunization using a novel lipid-based biphasic delivery system.

We have initiated the these experiments in mice and the results have been positive. We are currently designing new approaches to improve the responses and to transfer the technology to large animals.

Pasteurella haemolytica is a gram negative bacteria commonly isolated from the lungs of cattle with pneumonic pasteurelosis. Leukotoxin (Ltx) an exotoxin produced by *Pasteurella haemolytica* has been identified as a potential virulence factor, the recombinant protein produced in *E. coli* is currently used to immunize cattle to protect against fibrinous pneumonia.

In the present study we used Ltx and hen egg lysozyme as model antigens to investigate the ability of transdermal delivery of vaccine antigens to induce humoral (antibody) and cellular (antigen-specific proliferation and IL-4 secretion) responses in mice.

4.4.6. Assessment of immune responses induced by transdermal delivery of Ltx.

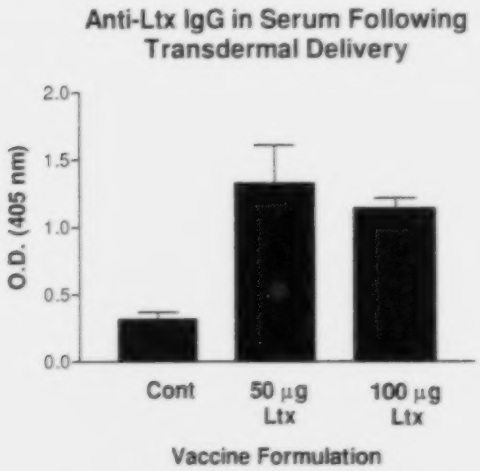

Fig. 6. Serum antibody responses following transdermal immunization with Ltx liposome patches. Mice were immunized by the transdermal route with 50 μg or 100 μg of Ltx, control animals received patches containing liposomes alone. The immunization was repeated on day 21 and the animals were bled 10 days later. Ltx-specific antibodies in serum were analyzed by ELISA and are expressed as optical density (O.D.) 405 nm at a serum dilution of 1:160.

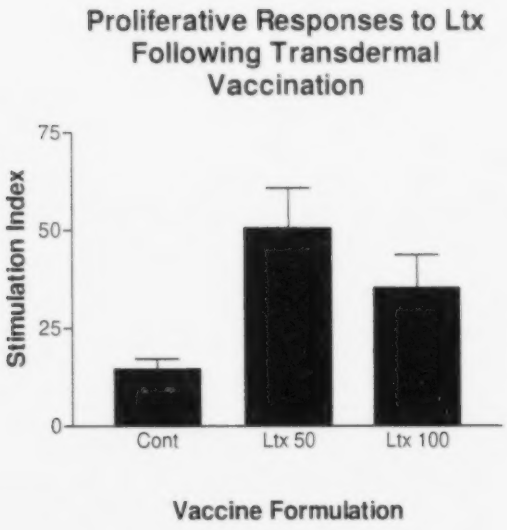

Fig. 7. Proliferative responses of spleen cells following *in vitro* stimulation with 25 μg/ml of Ltx. Spleen cells were obtained from mice immunized with 50 μg or 100 μg of Ltx, vaccination was repeated on day 21 and the animals were sacrificed 10 days later. Results are expressed as mean stimulation index and the bars indicate the standard deviation of three independent experiments.

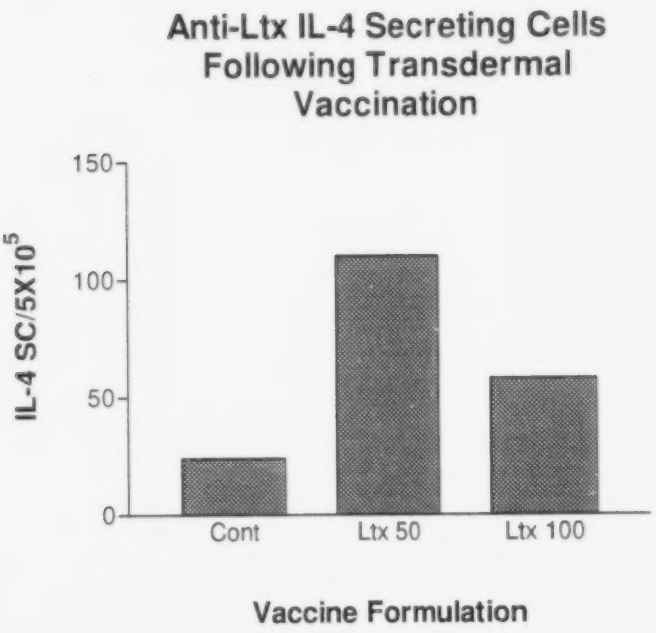

Fig. 8. Number of IL-4-secreting cells following *in vitro* stimulation of spleen cells with Ltx. Mice were immunized and boosted two weeks later. Spleen cells were harvested 10 days after the second immunization and cultured in the presence of Ltx for 48 hours prior to detection of cytokine. Results are expressed as the mean plus standard deviation of IL-4-secreting cells/$5X10^5$ cells of triplicate wells of pooled spleen cells from 4 mice.

4.4.7. Effect of transdermal delivery of Ltx on the induction of cell-mediated immunity measured by ELISOT assay for IL-4 and IFN-γ secreting cells.

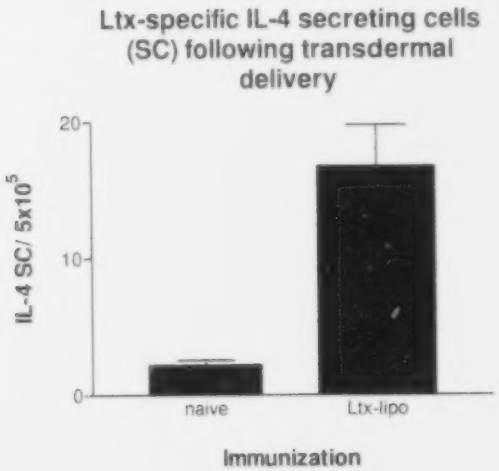

Fig. 9. To assess the type (Th1 vs Th2) and magnitude of cell-mediated immune responses induced by the transdermal administration of Ltx, the IL-4 and IFN-γ secreting cells were determined in spleens cells from immunized animals. The results demonstrated that the T-cell response was characterized by secretion of IL-4. No IFN-γ specific responses were observed.

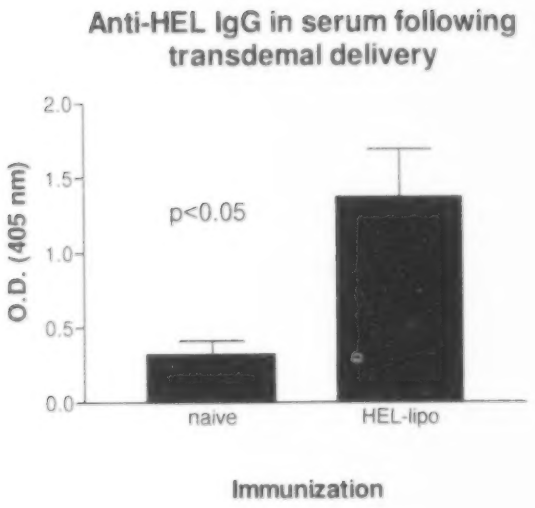

Fig. 10. Anti-HEL IgG response following transdermal administration of antigen. HEL alone or formulated in biphasic liposomes. The antibody levels were measured using an ELISA and the results expressed as the optical density of a 1:640 serum dilution of individual mice.

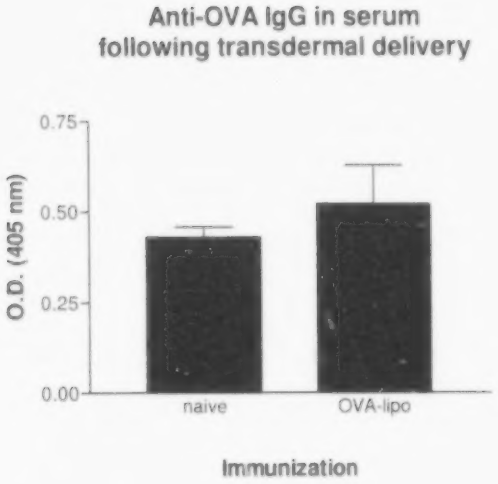

Fig. 11. Anti-OVA IgG response following transdermal administration. OVA was administered alone or formulated in biphasic liposomes. The antibody levels were measured using an ELISA and the results expressed as the optical density of a 1:640 serum dilution of individual mice. There was not significant differences between naïve and treated groups.

Cellular immune responses were measured by the spleen of naïve and immunized groups (HEL and OVA). No detectable antigen-specific IL-4 or IFN-γ secreting cells could be found in the spleen of these animals. The results of the Ltx experiment suggest that the cellular responses are mainly localized in the draining lymph nodes. Thus, it is important to assess the draining lymph nodes for the presence of antigen-specific T-cell responses in this model (OVA and HEL).

4.4.8. Oral delivery of model antigen by a novel lipid-based biphasic delivery system.

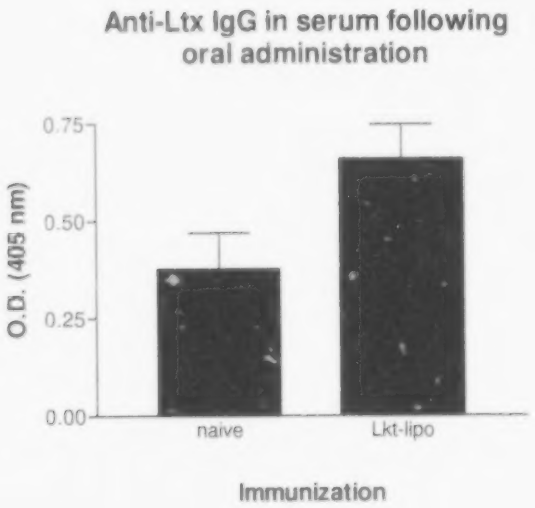

Fig. 12. The results of this experiment also showed that oral administration of biphasic liposomes containing a vaccine antigen appeared to induce anti-Ltx IgG. However, the immune response induced by this approach was not significantly different ($p=0.053$) from control animals. We believe, however, that oral delivery of vaccines using biphasic liposomes should be further examined.

4.4.9. Induction of HEL-specific antibody response in serum by transdermal immunization with HEL

We have previously shown that Lkt formulated in a Biphasix delivery system was capable of inducing humoral and cellular immune responses when delivered by the transdermal route. This experiment was designed to assess whether induction of immune responses by the transdermal route could be achieved with other proteins, and whether different formulations of the Biphasix delivery system influenced these responses. Hen egg lysozyme has been used extensively as a model antigen in the evaluation of immune responses in mice. Therefore, we selected HEL to evaluate the immune responses elicited following transdermal immunization. Topical administration of 50 μg of HEL formulated in various Biphasix compositions showed that formulation of the delivery system had an impact on the level of humoral and cell-mediated immune responses induced. All animals immunized with Biphasix delivery system developed HEL-specific IgG titres in the serum (Fig.13). However, there were significant differences between different formulations in their ability to induce HEL-specific IgG responses. Mice immunized with formulation V induced the highest levels of antigen-specific IgG in serum. The anti-HEL IgG response was characterized primarily by IgG1 subclass in all groups (Table 1). *In vitro* antigen stimulation of cells isolated from spleen and lymph nodes of immunized mice showed a predominant IL-4 response over IFN-γ (Fig. 14). Again, mice immunized with formulation V produced a higher proportion of IL-4 secreting cells when compared to the response of mice immunized with other formulations.

To determine whether the route of immunization influenced the immune response to HEL, mice were immunized with 50 μg of HEL formulated in the same delivery system by the subcutaneous and transdermal routes. Our observations indicated that the HEL-specific antibody response (Fig. 15) elicited by both routes of immunization were similar.

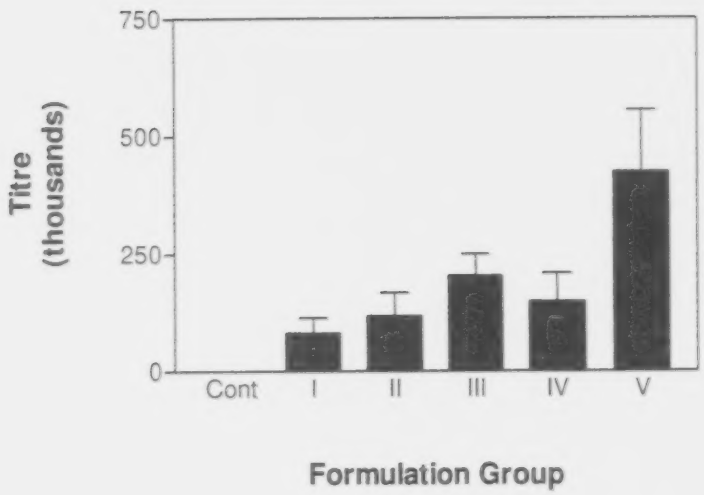

Fig. 13. HEL-specific IgG response in serum of transdermally immunized mice with 50 μg of HEL formulated in different Biphasix delivery systems. Data represent mean titres ± SEM of five individual mice.

4.4.10. Induction of HEL-specific IFN-γ and IL-4 secreting cells following transdermal immunization with different Biphasix formulations.

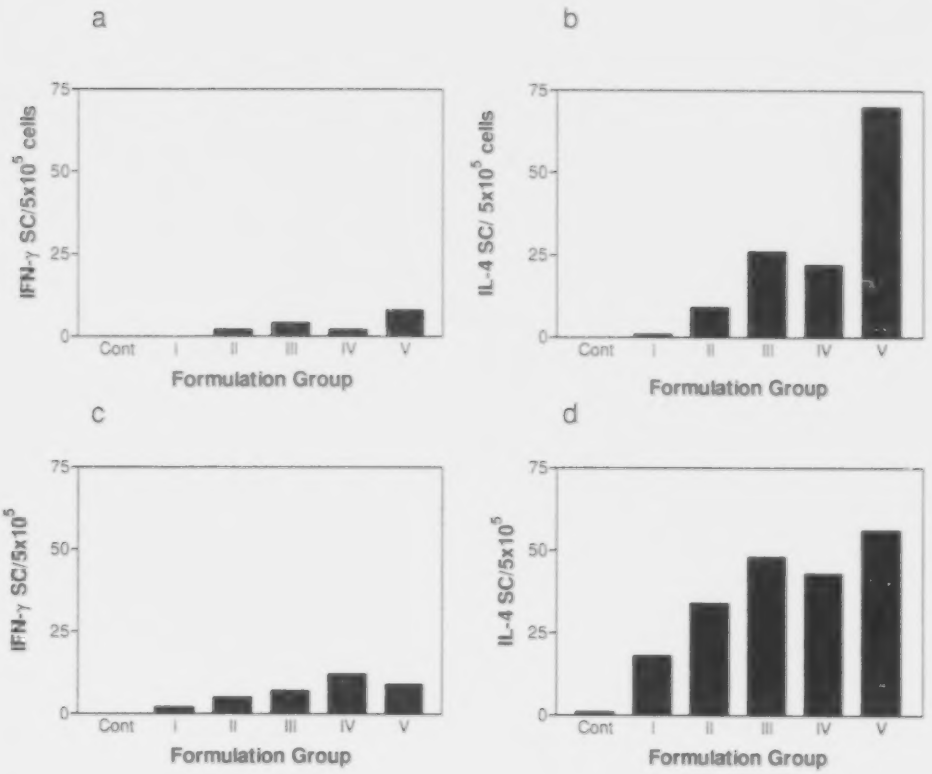

Fig. 14. HEL-specific IL-4 and IFN-γ secretion of spleen cells and draining lymph nodes cells following topical immunization with 50 μg of HEL formulated in different Biphasix delivery systems. Cells were cultured in the presence of 20 μg/ml of HEL and the frequency of IL-4 and IFN-γ-secreting cells in the lymph nodes (a and b) and spleen (c and d) was assessed by ELISPOT assay. Data represent the mean cytokine-secreting cells of pooled samples from five individual mice.

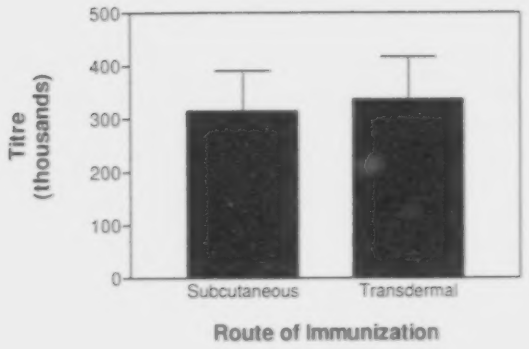

Fig. 15. Comparison between subcutaneous and transdermal routes in the induction of HEL-specific IgG in serum. Mice were immunized subcutaneously or transdermally with 50 μg of HEL formulated in the same Biphasix formulation and the levels of anti-HEL were determined in serum using an ELISA. Data represent mean titres ± SEM of five individual mice.

Table 1. HEL-specific IgG isotypes in serum following transdermal immunization with different Biphasix formulations.

Formulation	HEL-Specific IgG Isotype (GM)*			
	IgG1	IgG2a	IgG2b	IgG3
Control	<40	<40	<40	<40
I	402000	365	445	749
II	77040	1352	395	1681
III	728900	460	471	5964
IV	260400	490	1505	454
V	1335000	2036	3450	4379

* Titres are expressed as the geometric mean of 5 individual mice.

4.4.11. Assess the potential of transdermal vaccines carrying a protein antigen to induce mucosal and systemic immunity in cattle and sheep.

Following the successful induction of immune responses in mice using transdermal vaccines, our next objective for this period was to evaluate the effect of topical application of vaccines in sheep and cattle. We conducted a number of experiments to evaluate the thickness of the skin in different parts of the animal and to determine whether immune cells (Langerhans cells) were stimulated by the application of lipid-based formulations when applied to the skin.

Our results demonstrated that the permeability barrier of the skin in cattle and sheep can not be penetrated by the formulations used in mice. Therefore, we assessed whether potent enhancers of permeability could disrupt this barrier and allowed the induction of immune responses. The results of these experiments showed that some animals (20%) developed immune responses to the vaccine antigen, suggesting that the skin of sheep is equipped with immune cells capable of mediating the induction of immune responses.

At present, the barrier properties of sheep and cattle skin are not well understood. Reports in the literature suggested that drugs are much more likely to traverse sheep and cattle skins by way of skin appendages (i.e. hair follicles, sweat gland, and ducts) rather than through the cells of the stratum corneum. However, little is know about how drugs penetrate the skin of sheep and cattle. It is possible that one of the reasons why some pour-on formulations are very successful in penetrating the skin of sheep and cattle is because they use strong solvents. Strong organic solvents can dissolve the lipid-rich barrier of the skin and allow the penetration of drugs. Unfortunately, due to trait secret, the composition of most of the pour-on formulations have not been fully disclosed.

We have realized that in order to manipulate the permeability barrier of the skin of cattle we need to have a better understanding of the characteristics of the skin in terms of structure, immune competence and permeability. For these reasons we initiated some experiments with the aim of determining the structure and immune characteristics

presence of immune cells (Fig 1 and 2). These assays will allow us to evaluate the changes that occur in the immune cells of the skin following the application of different formulations. Because this assays can be done in the laboratory using skin form dead animals, this preliminary screening will be much less expensive than experiments using live animals.

As a continuation of the work presented in the last progress report, we have evaluated the effect of two immunomodulators (IL-12 and IFN-α).

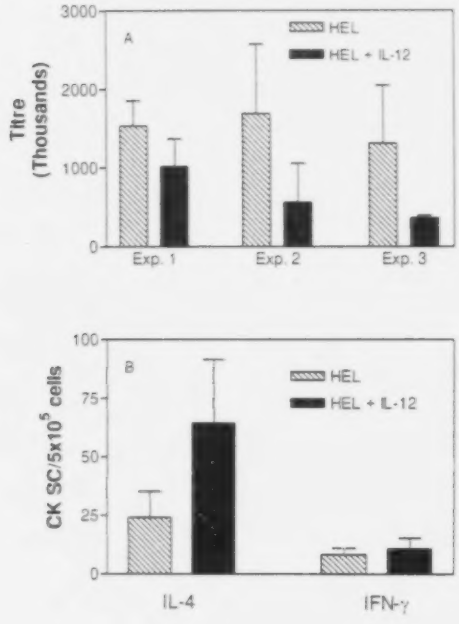

Fig. 16. To examine the effect of IL-12, a potent vaccine modulator, on the induction of humoral and cellular immune responses in mice. Co-administration of IL-12 and the antigen hen egg lysozyme (HEL) decreased the antibody response compared to the immune responses induced by administration of HEL alone (A). The cellular responses were not significantly changed (B). These results were different from our previous experiment using IL-12 as an immunomodulator for systemic and mucosal vaccines

(Baca-Estrada et. al. 1997 and Baca-Estrada et al. 1999). However, these experiments indicate that it is possible to modulate the immune responses by incorporating an immunomodulator into the vaccine formulation.

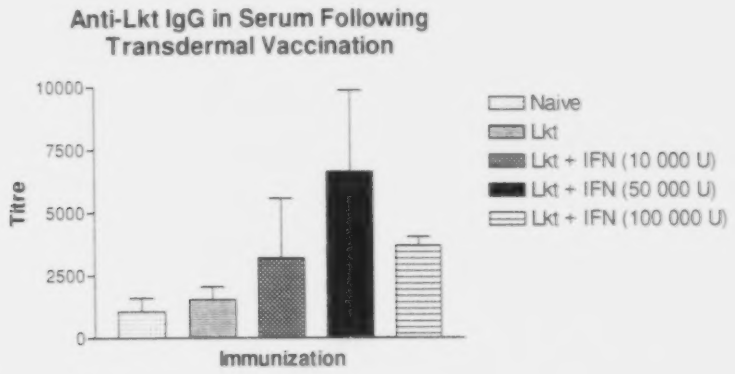

Fig. 17. Effect of IFN-α on the humoral immune response to Lkt following transdermal delivery. Mice were immunized with 10 μg of Lkt alone or together with different doses of IFN-α. The serum of immunized mice was analyzed by ELISA for the presence of anti-Lkt IgG. The results are expressed as the mean titre ± SD from 5 mice/group.

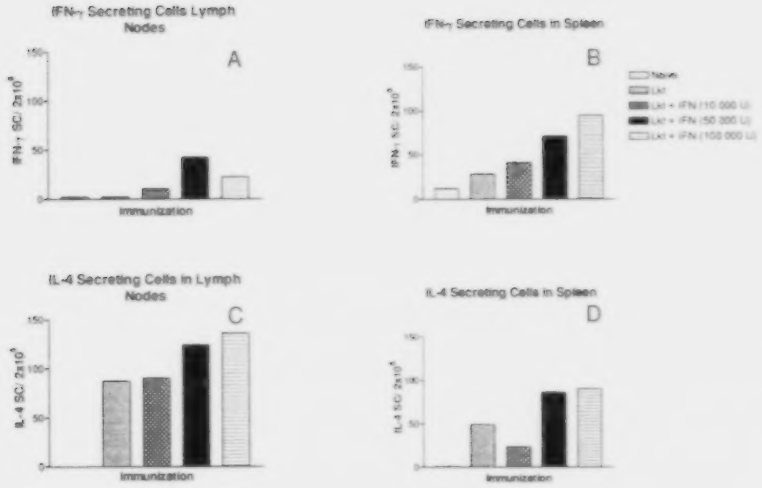

Fig. 18. Effect of IFN-α on the induction of cellular immune responses. Mice were immunized with 10 μg of Lkt alone or together with different doses of IFN-α. The Lkt-specific responses were measured in the spleen and lymph nodes of immunized mice using the IFN-γ (A and B) or IL-4 (C and D) ELISPOT assay. Results are expressed as the mean cytokine secreting cells (SC) of pooled samples from 5 mice/group.

4.4.12. Assess the potential of transdermal vaccines (protein and DNA) to induce protection against BHV-1 infection in cattle.

The assessment of transdermal vaccines in cattle to protect against disease has not been done due to the fact that the immune responses induced in large animals have been variable and low. Since challenge experiments in cattle are very expensive, we have decided to postpone these experiments until new formulations, more suitable for the penetration of the skin of cattle, are developed.

We have clearly demonstrated that lipid-based delivery systems can induce immune responses to protein vaccines when applied by the transdermal route in mice. We have also extended our observations indicate that these responses can be modulated by the addition of cytokines. We have initiated experiments to evaluate this vaccination approach in cattle by investigating the characteristics of their skin and to evaluate the incorporation of strong permiation enhancers and compounds that will allow a better penetration of the vaccines through the skin.

4.4.13. Assess the potential of transdermal vaccines carrying a plasmid DNA encoding a vaccine antigen to induce immune responses in cattle and sheep.

The development of delivery systems that can mediate transfection in vivo have not been fully characterized. Therefore, the testing of these formulations in cattle or sheep has been delayed. We carried out experiments to characterize these formulations in vivo. Five groups of BALB/c mice (three mice/group), were immunized either with 100 μg of gD encoding plasmid in a lipid-based delivery vehicle or with 1 μg gD encoding plasmid using a gene gun. Immunizations were administered 3 times at 2 week intervals. One day before each immunization mice were shaved and the skin left to recover before immunization. Two weeks after the last immunization gD specific humoral and cellular immune responses were tested using ELISA and ELISPOT respectively.

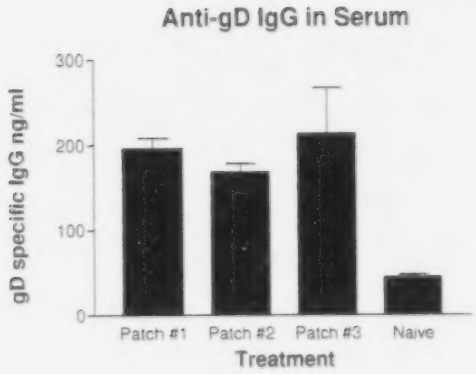

Fig. 19. This graph demonstrates the serum anti-gD IgG titres induced by transdermal delivery of plasmid encoding gD. ELISAs were done on individual mouse serum for total gD specific IgG. Although gD specific antibody was detected in all the immunized mice, gene gun immunized mice had much greater gD specific antibody 5000 ng/ml compared to 200 ng/ml obtained by mice immunized using a patch.

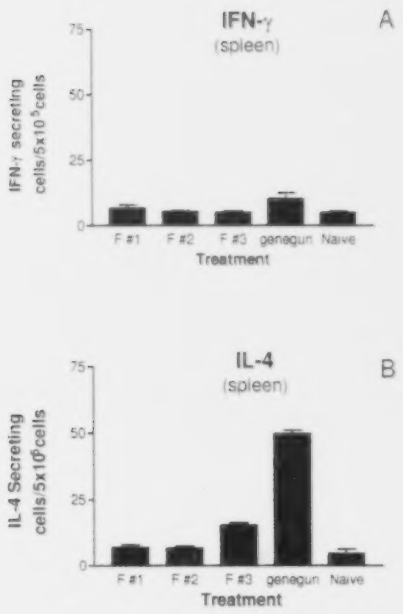

Fig. 20. This graph demonstrates the cytokine secretion of cells from transdermally immunized mice with a plasmid encoding gD. IFN-γ (A) and IL-4 (B) secreting cells were done using individual spleen cells. Cells were stimulated with 0.4 μg/ml of gD.

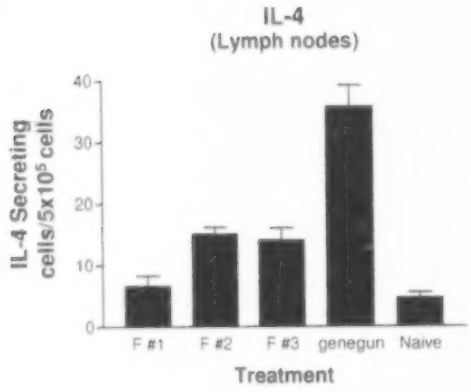

Fig. 21. This graph demonstrates the gD-specific IL-4 SC in draining lymph nodes of immunized mice. IL-4 SC were found in gene gun immunized mice as well as in some groups immunized with the lipid-based formulations. No IFN-γ SC were detected in any group.

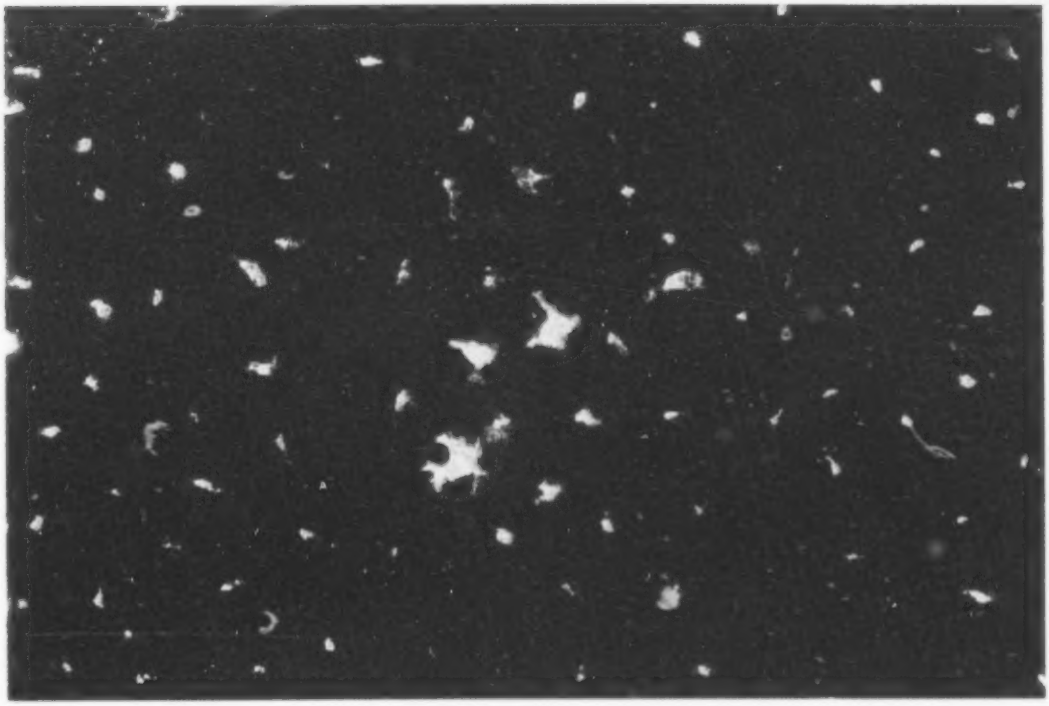

Fig. 22 . Immunofluorescence staining of MHC class II positive cells in epidermal sheets. The epidermis was separated from the dermis by incubating the tissue (dermal side down) with PBS containing 20 mM EDTA at 37°C for 3 hours. Epidermal sheets were peeled from the underlying dermis, by holding the dermis with fine forceps and pulling the epidermis from the edge with another set of forceps. Isolated epidermal sheets were fixed in acetone for 20 min at room temperature. Sheets were incubated with FITC-labeled antibody overnight at 4°C. Specimens were placed on glass microscope slides, air dry, then mount under glass coverslip with appropriate mounting reagent.

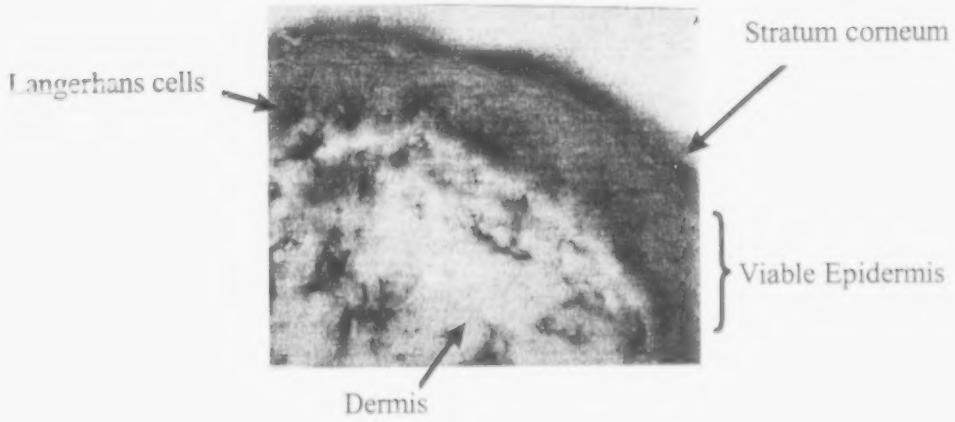

Fig. 23. Immunohistochemical staining of normal pig skin with anti-MHC Class II. Cryosections of pig skin were stained with a pooled monoclonal antibodies specific to MHC Class II (VMRD, Inc. Pullman, WA) at a dilution of 1:1000 followed by incubation with goat anti-mouse IgG HRP labeled. Langerhans cells are observed at the basal layer of the epidermis.

The assessment of transdermal vaccines in cattle to protect against disease has not been done due to the fact that the immune responses induced in large animals have been variable and low. Since challenge experiments in cattle are very expensive, we have decided to postpone these experiments until new formulations, more suitable for the penetration of the skin of cattle, are developed.

4.5. Conclusions

- Formulations capable of stimulating immune responses in mice following topical application of plasmid DNA have been developed.

- Transdermal immunization of mice with HEL formulated in a Biphasix delivery system induces cellular and humoral immune responses. Our results demonstrated that the immune response was of a Th2 type characterized by the predominant induction of antigen-specific IgG1 antibody in serum and IL-4-secreting cells in the spleen and lymph nodes.

- The induction of immune responses to the antigen are influenced by the formulation of the delivery system. Our results suggest that optimal Biphasix formulations need to be established for each vaccine antigen to induce the most effective immune response.

- Transdermal delivery of a bacterial antigens such as Ltx induces antigen-specific humoral and cellular responses. The cellular response is characterized by the predominant induction of antigen specific IL-4 over IFN-γ. The proportion IL-4 secreting cells was higher in the draining lymph nodes than in the spleen of immunized mice.

- Topical application of HEL formulated in biphasic liposomes induced significant anti-HEL IgG.

- Other antigens such as gD (4 and 40 μg) and OVA (100 μg) were used. No antigen-specific immune responses were detected in animals receiving gD or OVA at these tested concentrations.

- Methods to evaluate the penetration of vaccines through the skin and activation of immune cells (Langerhans cells) have been developed.

- Immunomodulation of immune responses induced by transdermal vaccines (protein) have been observed using IL-12 and IFN-α.

- Transdermal delivery of HEL in Biphasix formulations induces similar levels of specific IgG antibodies in serum than subcutaneous immunization with the same formulation.

- We have clearly demonstrated that lipid-based delivery systems can induce immune responses to DNA vaccines when applied by the transdermal route in mice.

5. Information of Benefit to Producers

The potential of developing cost effective vaccine delivery systems such as liposomes provides an opportunity to enhance disease control and safety over currently available vaccines. Preventing disease with better vaccines should improve farm efficiency and profits as well as resulting in consumer savings. Added benefits may be incurred through the reduction of carcass trim and hide damage due to reactive vaccines and excessive antibiotic treatment. VIDO continues to update producers groups on the results of this research on an ongoing basis.

6. Personnel

Dr. Maria Baca-Estrada - 100% Research Scientist

Catharine Ewen 100% Technician.

Marlene Snider 100% Technician.

Shawn Babiuk 100% Graduate Student.

I here by certify that the above research has been completed.

Maria E. Baca-Estrada

Research Scientist